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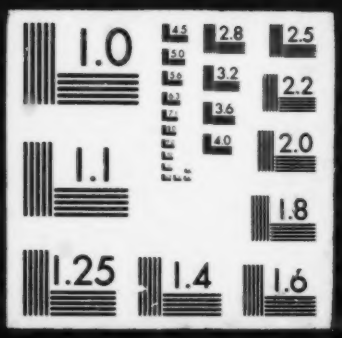

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CONSTRUCTION AND UTILIZATION OF A SIMPLE DEVICE FOR THE PRODUCTION OF REACTIVATABLE PHOSPHATASE

C. B. Donnelly, et al

National Center for Urban and Industrial Health
Cincinnati, Ohio

1967

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A SIMPLE DEVICE FOR THE PRODUCTION OF REACTIVATABLE PHOSPHATASE

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U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
Public Health Service

22

CONSTRUCTION AND UTILIZATION OF A SIMPLE DEVICE FOR THE PRODUCTION OF REACTIVATABLE PHOSPHATASE

C. B. Donnelly, J. E. Leslie,
J. D. McDaniel, and L. A. Black

Details of illustrations in
this document may be better
studied on microfiche.

U. S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
Public Health Service
Bureau of Disease Prevention and Environmental Control
National Center for Urban and Industrial Health
Cincinnati, Ohio

1967

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ABSTRACT

A laboratory-scale device for the heat-treatment of cream, with a capacity of 5 liters per hour, is described. The apparatus consists of a heating coil of small-diameter nylon tubing immersed in a constant-boiling liquid bath, through which milk or cream can be forced at a constant flow rate by air pressure. Cost of materials and labor is estimated at $80.00. Cream heat-treated in the device contained reactivatable phosphatase that was suitable for testing an analyst's ability to detect reactivated phosphatase. Such heat-treated products have been used successfully in a national split-sample program.

CONSTRUCTION AND UTILIZATION OF A SIMPLE DEVICE FOR THE PRODUCTION OF REACTIVATABLE PHOSPHATASE

INTRODUCTION

With increasing use of ultra high temperatures for the pasteurization of milk and milk products, the phenomenon of incomplete denaturation and partial reactivation of the alkaline phosphatase enzyme of milk has become of practical interest in the dairy industry. The presence of phosphatase has been accepted as a positive indication that milk was not subjected to sufficient heat for sufficient time to destroy most microorganisms of public health significance, or it had been mixed with raw milk. Under conditions of ultra high temperature (UHT) pasteurization, phosphatase is reversibly inactivated, and it may be partially reactivated if the milk is stored at elevated temperatures. If reactivation in a dairy product pasteurized by a UHT process has occurred, application of the standard phosphatase tests could lead to a false conclusion that the product had been improperly pasteurized.

Methods for laboratory preparation of milk and cream that contain reactivatable phosphatase have been published previously by Diehl, Thomas, and Campbell (4); McFarren, Black, and Campbell (7); and Jung and Sanderson (5, 6). A research type of apparatus with coils of stainless steel tubing is expensive, however, and cannot be fabricated readily by state laboratories that may need to prepare split samples containing reactivatable phosphatase for testing by local laboratories. Our purpose has been to produce at minimum cost and with minimum fabrication needs, a useful apparatus for the heat-treatment of cream containing reactivatable phosphatase, primarily for use by state milk laboratory approval agencies.

This apparatus (Figures 1 to 4) substitutes relatively inexpensive nylon tubing, which can be used and discarded, for stainless steel in the heating coil and thus eliminates difficult cleaning; and a constant-boiling liquid bath is used for temperature control, in place of a regulated heater. Materials and fabrication are relatively simple and inexpensive, and are estimated to cost less than $80.00, which is a fraction of the present cost of a suitable electrically-controlled constant-temperature bath.

Figure 1. Heat-treatment device.

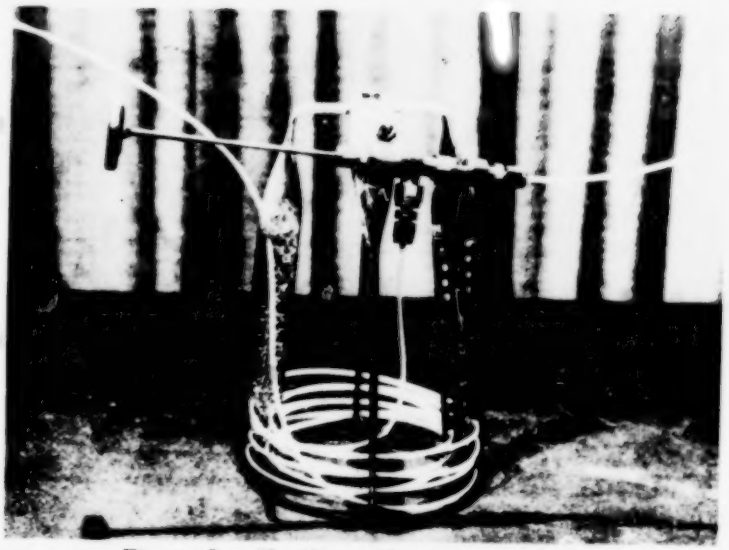

Figure 2. Heating coil on support form.

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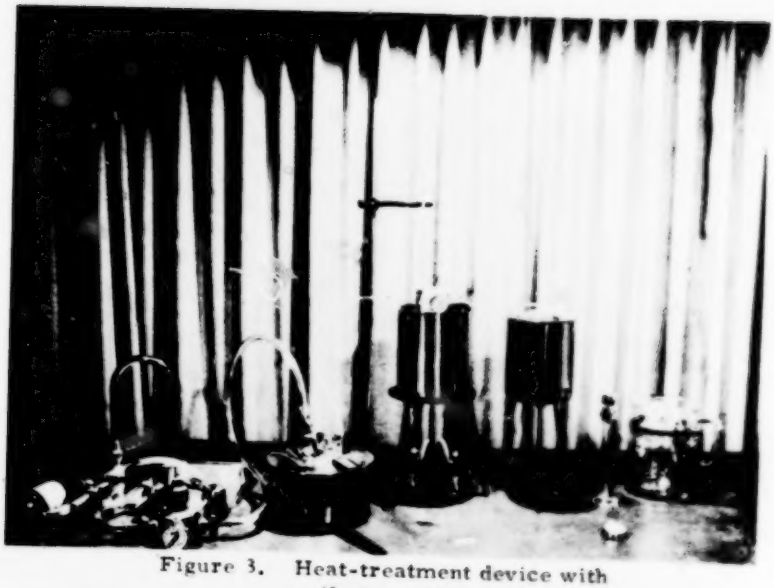

Figure 3. Heat-treatment device with coils in position.

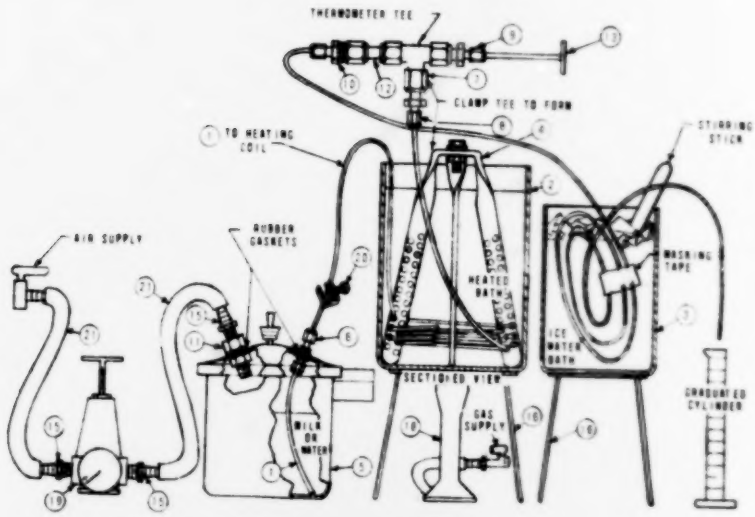

Figure 4. Diagram of heat-treatment device; key to list of materials.

MATERIALS*

Item

1. Twenty-two feet of 1/8-inch outside diameter (O. D.) and 0.078-inch inside diameter Type H nylon tubing.

2. One metal beaker of at least 3,500-ml capacity for heating bath.

3. One metal beaker of approximately 3,000-ml capacity for cooling bath.

4. A form, grid, or screen for supporting the coil of tubing, which will provide at least a 1/4-inch pitch of the tubing, and will fit inside item 2.

5. One stainless steel 4-quart 15 psi (or more) pressure cooker.

6. One "Swagelok" stainless-steel bulkhead union for 1/8-inch O. D. tubing with all front and rear ferrules made of nylon or teflon.

7. One "Swagelok" stainless-steel all tube tee for 1/4-inch O. D. tubing with stainless-steel front and rear ferrules.

8. One "Swagelok" reducer 1/4- to 1/8-inch O. D. tubing, stainless steel, with front and rear nylon or teflon ferrules.

9. Same as above, but 1/8-inch hole, and nylon or teflon ferrules must be bored to 0.147 inch to admit the stem of a Weston† metal thermometer.

10. One "Swagelok" reducing union from 1/4- to 1/8-inch O. D. tubing, stainless steel with 1/8-inch O. D. nylon or teflon front and rear ferrules.

11. One "Swagelok" female bulkhead connector with 3/8-inch female pipe thread made of brass.

12. One piece of 1/4-inch O. D. stainless-steel tubing 1.6 inch long.

*Except where otherwise noted, item numbers correspond to numbers in Figure 4.

† Mention of commercial products does not imply endorsement by the Public Health Service.

13. One Weston all metal stainless-steel thermometer that should read up to 220°F.

14. One mercury thermometer, up to 250°F (shown in Figure 1).

15. Three 3/8-inch pipe thread male hose connectors.

16. Two tripods, or one tripod and one block of equivalent height.

17. One ring stand (shown in Figure 1).

18. Two Fisher or Meker burners.

19. Pressure regulator, Norgren type 11-006, 0-40 psig with gauge and 3/8-inch pipe size, or one providing equivalent accuracy.

20. Hose clamp, preferably with a fine thread, or valve adapted to nylon tubing.

21. Two pieces of 1/2-inch O. D. tygon or rubber tubing.

22. Calcium chloride, 45 percent solution: 3 pounds $CaCl_2$ anhydrous (drying grade is satisfactory) dissolved in water and made to 3 liters. (Not shown in Figure 4.)

Miscellaneous items: Water, ice, salt (NaCl), stirring stick, waste receiver, milk-receiving flasks in ice baths, air outlet, gas outlet, clamp to put item 7 on item 4, 100-milliliter graduated cylinders, rubber gaskets, asbestos gloves.

FABRICATION

Pressure Cooker. Drill a 3/4-inch hole for item 11 midway between center of pressure cooker lid and edge. Make or buy a gasket that will just fit over the male end of item 11. Insert item 11 into lid, thread on nut, and make air-tight. Thread one hose connection (item 15) into item 11. Drill a 5/16-inch hole midway between center of pressure cooker lid and edge. Make or buy a gasket concentric to this hole. Insert item 6 into this hole in lid, thread on nut, and make air-tight.

Air Regulator. Tighten (with wrench) the remaining two hose connectors (item 15) into each end of item 19.

Tubing Form, item 4. Make 1/8- or 3/16-inch-thick by 3/4-inch-wide strips of aluminum. Make top smaller than bottom and make long enough so that top stands 2 inches above top of

liquid bath. Drill a minimum of 15 3/16-inch holes in each leg, and space about 1/2 inch apart alternating from edge to edge. Bend to shape in a vise, bolt together, and fit into beaker (item 2). File bottom of legs as necessary until form rests evenly on bottom of beaker (item 2).

Temperature Tee. Bore one reducer nut (item 9) to enlarge its diameter from 1/8 inch to 0.147 inch (or slightly larger). Ream, drill, or bore nylon front and rear ferrules to enlarge them from 1/8 inch to 0.147 inch (or slightly larger). Slide nut, front ferrule, and rear ferrule (in that order) onto thermometer stem (item 13) at a position such that front of front ferrule is 3.575 inches from end of stem. Thread nut onto reducer, give one-fourth turn with wrench, and insert reducer (item 9) into one flow-through end of item 7. With item 9 seated in item 7, tighten nut on item 7 by hand and then give an additional one-and-one-quarter turn with wrench. Item 7 should grip item 9 securely. Insert item 8 into angle leg of item 7 until it bottoms. Tighten nut on item 7 by hand, then give an additional one-and-one-quarter turn with wrench. Take item 12, file ends smooth and square, and insert into remaining flow-through leg of item 7 until it bottoms. Tighten nut by hand and give an additional one-and-one-quarter turn with a wrench. Take item 10, push onto item 12, and repeat above tightening procedure.

ASSEMBLING APPARATUS

Pressure Feed Reservoir. Cut a length of tubing (item 1) that will fit between pressure cooker (item 5) bottom and bulkhead union (item 6). Put tubing in union until it bottoms, and tighten as much as possible with fingers; further tightening will constrict the tubing and reduce flow. Fill pressure cooker three-quarters full with water, and close lid. Force one length of rubber hose or tygon tubing (item 21) over hose connectors between air supply and pressure regulator, and another length between pressure regulator (item 19) and hose connector (item 15) on pressure cooker.

Heating Coil. Cut a 13-foot length of tubing (item 1), and mark it at a point 2 feet from one end. Insert this end into the bulkhead union (item 6), and make the nut finger-tight. Weave tubing onto form by starting 1/2 inch from bottom and progressing upward. Two-foot mark on tubing should just enter bath at surface of boiling liquid. Other end of tubing should enter angled leg of tee when temperature tee is in clamped position on form. Nut on item 8 should be made finger-tight. Avoid kinking the tubing with tight radii.

Hold Coil. Initially, a "hold" coil was included in the set-up. Leading from the flow-through leg of the thermometer tee, 2-foot nylon tubing measured off from the end of the cooling coil was wound on the support for immersion in the boiling bath along with the heating coil; however, a product of equal quality was obtained when the holding coil was omitted, and the flow was directly from the thermometer tee to the cooling coil, with only the length of tubing necessary to bridge the distance from the boiling bath to the cooling bath (about 1 foot) serving as a "hold."

Heating Bath. Fill metal beaker (item 2) with about 3 liters of 45 percent calcium chloride solution (item 22), and heat on tripod (item 16) over burners (item 18). The large heat transfer involved requires a heating bath volume of 3 liters or more, and two Meker or Fisher type gas burners. Boiling-point temperature may be adjusted by adding more $CaCl_2$ or water. For determining bath temperature, suspend a mercury thermometer from a ring stand, and position it in the heating bath so that its immersion mark is about level with the surface of the boiling liquid (Figure 3). Replace water lost by evaporation in small increments to maintain the level at the immersion mark without cooling the bath.

Clamp or bolt temperature tee (items 10, 12, 8, 7, 9, and 13) to support item 4 in such a position that the thermometer will register the true temperature of the milk passing through the tee. The location of the thermometer tee will affect the registered temperature. If located too near the boiling liquid, or where it receives direct heat from the burners, the tee itself will be overheated; however, locating the tee too far away from the heating bath results in heat loss, and insulation of the tee results in a lag in response. In the set-up described, the horizontal arm of the tee is about 2 inches above the surface of the boiling liquid, and away from the heat blast from the burners. Our experience has been that the thermometer registered appropriate boiling-point temperatures when the flow of either water or milk was reduced to the rate at which boiling started within the heating coil.

Ethylene glycol in water was used initially to prepare the constant boiling mixture for the heating bath, but later was rejected because of its toxic vapors. In subsequent use, both water boiling at 211° to 212°F, and 45 percent $CaCl_2$ (w/v) in water boiling at 236° to 238°F gave satisfactory results(Table 1).

Table 1. TEMPERATURES[a] WITH CONSTANT-BOILING BATH MIXTURES

Bath mixture	Boiling temperature, °F	Milk temperature, °F[b]
Distilled water	212	198
NaCl solution 12% w/v[c]	216	n.d.[e]
NaCl solution saturated[c]	226	n.d.[e]
Ethylene glycol solution 50% v/v[d]	228	208
Ethylene glycol solution 60% v/v[d]	236	218
$CaCl_2$ solution 35% w/v	228	208
$CaCl_2$ solution 38% w/v	230	n.d.[e]
$CaCl_2$ solution 41% w/v	233	n.d.[e]
$CaCl_2$ solution 45% w/v	236	218

[a] Temperatures varied 1 to 6 degrees with changes in composition of bath by evaporation.
[b] Milk temperature at a flow rate of 88 milliliters per minute.
[c] Corrosive.
[d] Prestone; fumes are toxic.
[e] Not determined.

Cooling Coil and Bath. Cut a 9-foot length of tubing (item 1). Insert one end into the flow-through leg of the temperature tee (nut made finger-tight). Allow about 1 foot to bridge the gap between the heating bath and the cooling bath. Coil loosely the remaining length, leaving about 6 inches to reach into the receiving flask, and fasten on one side with masking tape, for immersion in the ice bath after flow has been started. Prepare salt-ice-water baths for the cooling coil (item 3) and also for the receiving flask (temperature 26° to 28°F). If possible, place an asbestos-board shield between the heating bath and the cooling bath. Constant vigorous agitation of the cooling bath is necessary to make the milk sensibly cool as it emerges from the coil. As with the heating bath, the large quantities of heat exchanged require almost constant replenishment of ice and salt, with frequent drain-off of the melt water.

METHOD OF OPERATION

Adjustment and Equilibration with Water. When the system has been assembled as described in "Assembling Apparatus," with water in the pressure cooker and the constant boiling bath stabilized at about 236°F (in the range 234° to 238°F), place a waste receiver under the outlet end of the cooling coil and place the pressure cooker pressure regulator over the vent pipe. Close the line pressure by turning pressure regulator handle counter clockwise until there is no more resistance to turning (disengaged). Open cock to air line. Turn handle of pressure regulator clockwise, slowly, until gauge reads about 12 psi. In a few seconds, water should issue from the spout into the waste receiver. Place the heating coil in the boiling bath and the cooling coil in the ice bath. If boiling decreases or stops, turn up flame on burners. Place clamp (item 20) onto tubing about 2 inches above bulkhead union on pressure cooker, and tighten until flow is visibly decreased. Measure the flow rate with a graduated 100-milliliter cylinder. Adjust the clamp to give a stable flow rate of about 78 milliliters of water in 30 seconds. The temperature of the water at the thermometer tee should stabilize at about 175°F, and the bath should continue to boil vigorously.

Heat-Treatment. After the rate of flow is adjusted with water, remove both coils from baths, and close the gas cock on the air line. Bleed off the air pressure in the pressure cooker by slowly lifting and then completely removing the pressure regulator on the cooker from the air vent. Open the cooker, remove the water, and fill cooker to four-fifths full with milk or cream. Close the pressure cooker, and replace the regulator on the air vent. Replenish ice if necessary. When the bath is boiling, open the airline cock, which will result in a flow of milk or cream. Place supporting form in heating bath and cooling coil in ice bath, and determine for 30 seconds the flow rate using a graduated cylinder. Adjust flow to about 88 milliliters per minute. At this flow rate, the heating time will be about 7 seconds and the "hold time" will be about 0.8 seconds. The temperature of milk or cream at the thermometer tee should stabilize in the range of 212° to 218°F, with a flow rate of 88 milliliters per minute and a bath temperature of 236°F (flow rate may be varied slightly to give desired temperature). Allow about 100 milliliters to pass to waste. To take a sample, place the receiving flask in an ice bath under the discharge end of the cooling coil. Stir the ice-water bath containing the cooling coil vigorously during the entire time of sample collecting. At 88 milliliters per minute, pasteurizing 1 liter requires 11.4 minutes. Monitor the boiling bath temperature and the milk temperature. After sufficient

sample has been collected, remove the receiver, and again measure the flow rate. Remove burners and coils from baths; let the milk run for a minute to cool the tubing, and then close the air line. Slowly remove the pressure regulator on the cooker, open the cooker, and empty the contents. Rinse cooker, refill with water, replace in train, and force water through the coils to rinse. The same coil may be reused without disassembly over several days if the thermometer tee is cleaned and the apparatus is rinsed thoroughly with water. The calcium chloride solution may also be reused.

When the heat-treatment is completed, loosen all nuts holding tubing, and throw the nylon tubing away being careful to retrieve all nylon ferrules. The bulkhead union and thermometer tee should be cleaned.

RESULTS AND DISCUSSION

Heat-Treatment of Milk and Cream and Reactivation. Twenty samples of milk and cream were processed with this apparatus. The butterfat content was varied from 3.5 to 20 percent, and nominal pasteurization temperatures of 198°, 208°, and 218°F were tried. The rate of flow, measured with a stop watch and a graduated cylinder at the outflow end of the cooling coil, was held constant as nearly as possible at 88 milliliters per minute.

The heat-treated samples were divided into several portions, some of which were held at temperatures of 25°, 30°, or 35°C for periods of 15, 30, 45, 60, 120, and 240 minutes to permit reactivation. This was done to determine the optimum conditions for reactivation of a product, which would be suitable for split samples. This requires that the sample yield a positive phosphatase test upon initial testing, and that, upon application of the differential test, reactivated phosphatase be demonstrated.

Portions of unreactivated and reactivated samples were tested for phosphatase on the day they were prepared and again on 3 succeeding days to determine their keeping quality. All samples were stored at 38° to 40°F when not in use.

Phosphatase Tests. Tests for residual phosphatase were made by the Scharer I (rapid) method (1) and by the modified spectrophotometric laboratory method (7). Differential tests for residual and reactivated phosphatase were made by the method of Campbell and McFarren (3, 2), and portions of each aliquot were tested by both the Scharer I and the modified spectrophotometric method.

Pasteurization efficiency of the apparatus was high. Standard plate counts (SPC) (32°C) after heat-treatment were less than 300 per milliliter (Table 2), and residual phosphatase tests were negative. After reactivation, counts were still less than 300 per milliliter, and tests for microbial phosphatase were negative.

Table 2. PASTEURIZATION EFFICIENCY: SPC/ml, 32°C, OF 20% CREAM

Raw cream SPC/ml	Heat-treated cream Temp, °F	Heat-treated cream SPC/ml	Cream reactivated 60 min at 25°C SPC/ml
20,000,000	208	20	30
250,000	197	40	9
250,000	215	15	8
4,400,000	198	230	220
4,400,000	218	15	7

Standardized raw milk, 3.5 percent butterfat, heat-treated with this apparatus, contained small amounts of reactivatable phosphatase, but less than could be detected reliably by the commonly used Scharer rapid methods. Cream with 10 to 20 percent butterfat, after heat-treatment, contained three to five times more reactivatable phosphatase, which persisted for at least 4 days with adequate cold storage, both before and after reactivation (Tables 3 and 4). Of the several kinds of milk or cream

Table 3. REACTIVATION AS A FUNCTION OF BUTTERFAT CONTENT[a]

Butterfat, %	Phosphatase in raw product, µg phenol/ml	Reactivated phosphatase in UHT heat-treated product, µg phenol/ml	Reactivation, %
3.5	4,000	32	0.8
10	6,700	93	1.4
14	5,900	90	1.5
20	7,500	182	2.4

[a] Values are averages of several determinations.

Table 4. STABILITY AT 40°F OF PHOSPHATASE VALUES FOR HEAT-TREATED 20% CREAM AS DETERMINED BY THE MODIFIED SPECTROPHOTOMETRIC LABORATORY METHOD

Reactivation	Freshly heat-treated			Stored 4 days at 40°F		
	Initial test	Differential test		Initial test	Differential test	
		No additive, undiluted	+$MgCl_2$, diluted 1:6		No additive, undiluted	+$MgCl_2$, diluted 1:6
	μg phenol/ml					
None[a]	0.3	22	32	0.6	19	33
25°C 30 minutes[a]	6	22	32	7	21	32
25°C 60 minutes[a]	11	24	31	12	24	28
25°C 15 minutes[b]	3	23	31		(not determined)	
25°C 15 minutes[b] +0.1% raw milk	10	20	27	9	24	25

[a] Average for five samples, determined from averages of optical densities.

[b] One sample, 1965 No. 7, stored 5 days at 40°F.

tried, cream containing 20 percent butterfat consistently gave the best results and is recommended for use in split samples. For use with 20 percent cream, all three temperature levels tried (198°, 208°, and 218°F) appeared to be about equally efficient (Table 5). Reactivatable phosphatase in the heat-treated product varied from 1.5 to 3 percent of the phosphatase initially present in raw cream, with a mean value of 2.4 percent. The level of reactivatable phosphatase was more directly related to the initial phosphatase content of the raw sample than to differences in heat-treatment temperature (within the range 197° to 218°F) or to differences in holding time (0.8 or 2.6 seconds).

Table 5. REACTIVATION AS A FUNCTION OF INITIAL PHOSPHATASE CONTENT IN 20% CREAM

Heat-treatment temperature, °F	Hold time, sec	Phosphatase in raw cream, µg phenol/ml	Reactivated phosphatase,[a] µg phenol/ml	Percent reactivation
208	2.6	6,750	105	1.6
208	2.6	6,500	204	3.1
208	2.6	10,500	222	2.1
197	<1	6,500	162	2.5
198	<1	8,250	174	2.1
215	<1	6,000	186	3.1
218	<1	8,500	210	2.5
218	<1	7,000	171	2.4
	Mean:	7,500	179	2.4

[a] Modified spectrophotometric laboratory method, $MgCl_2$ added.

Reactivation at temperatures of 30° and 35°C resulted in a rapid loss of the differential characteristic. Reactivation at 25°C for 15 minutes did not reliably give a product that was positive upon initial testing by the Scharer rapid method; reactivation at 25°C for 60 minutes or longer led to a less definite differential test. Holding at 25°C for 30 to 45 minutes appeared to be best for preparing samples that would be positive for phosphatase upon initial testing and clearly positive for reactivated phosphatase in the differential test. With all preparations, incubating the sample to the degree necessary to give a positive phosphatase test by the Scharer I method resulted in a decreased difference in the differential test. For our own use in split samples shipped across the country under less than ideal temperature conditions,

we partially reactivated the heat-treated cream and then added raw milk to ensure a positive initial phosphatase test (Table 6).

Table 6. PHOSPHATASE TESTS OF HEAT-TREATED 20% CREAM, 3 DAYS OLD, WITH ADDED RAW CREAM, AND WITH REACTIVATION AT 25°C

Raw cream added, %	Spectrophotometric method (8)			Scharer I method (1)		
	Initial test	Differential test		Initial test	Differential test	
		No additive, undiluted	+$MgCl_2$, diluted 1:6		No additive, undiluted	+$MgCl_2$, diluted 1:6
	μg phenol/ml			units/ml		
0.0	0	25	36	< 1	> 2 < 5	> 5
0.1	8	31	36	> 1 < 2	5	> 5
0.2	13	31	36	> 2 < 5	5	> 5
Time at 25°C, min						
0	1	25	35	< 1	> 5	> 5+[a]
30	8	25	32	> 1 < 2	> 5	> 5+[a]
45	11	28	34	> 2 < 5	> 5	> 5+[a]
60	13	30	35	> 2 < 5	> 5	> 5+[a]

[a] Differential test was positive in direct comparison.

Application in Split-Sample Program. Cream containing 20 percent butterfat heat-treated with the apparatus was used in the 1965 and 1966 split-sample programs* conducted by this Center and participated in by official laboratories of all states.

In 1965, samples 7 and 8 were examined for reactivated phosphatase. The base of both samples was 20 percent cream heat-treated in the laboratory at 218°F with < 1-second hold. The portion for sample 7 was reactivated by holding at 25°C for 15 minutes; in addition, 0.1 percent raw milk was added to provide an initial positive test. Sample 8 was reactivated at 25°C for 45 minutes.

In 1966, four samples were examined for reactivated phosphatase. Two were sterilized 20 percent cream; to one of these, raw milk was added. Two were prepared to contain reactivatable phosphatase (Table 7).

* Participants prepared the differential tests with smaller volumes of sample (4 ml) than those specified by McFarren (3).

Table 7. PHOSPHATASE TESTS OF FOUR 20% CREAM SAMPLES - 1966 SPLIT SAMPLES

Sample No.:		15	16	17	18
Sample composition:					
Heat-treatment temperature, °F		--	--	212-218	212-218
Reactivation		--	--	25°C 30 min	25°C 45 min
Sterilization		115°C 10 min	115°C 10 min	--	--
Raw milk added, %		0.3	--	0.1	0.1
Reported findings:					
No. of labs reporting	Sample temperatures, °F[a]				
42	33-50; mean 41	Residual	Negative	Reactivated	Reactivated
3	36, 40, 40	Residual	Negative	Residual	Reactivated
2	40, fr	Residual	Negative	Reactivated	Residual
4	52, 54, fr, fr	Residual	Negative	Residual	Residual
1	42	Residual	Residual	Reactivated	Reactivated
3	40, 42, 45	Reactivated	Negative	Reactivated	Reactivated
1	fr	Reactivated	Negative	Residual	Reactivated
1	fr	Negative	Negative	Reactivated	Reactivated

[a] When received by participating laboratories; fr = frozen sample.

In the split sample series of 1965, analysts in 39 of 57 participating laboratories successfully detected reactivated phosphatase in both samples. In 1966, analysts in 45 of 57 participating laboratories reported correctly a set of four samples (Table 7). In both years, several of the laboratories from which reports were at variance with the composition of the samples reported also that the temperature of the samples as they were received was above 40°F; in these instances, reactivation may have proceeded beyond the levels at which the differential test can be applied.

The necessity for holding the samples at a low temperature is emphasized. Use of small quantities of sample and reagent in preparing the differential test was apparently satisfactory. The precaution of Jung and Sanderson (6) in preparing differential tests should be heeded: place the small quantity of magnesium chloride solution in the tube before adding the sample.

The integrity of frozen samples was cursorily explored in both years; in 1966, when inclement weather delayed delivery of the main samples to some participants, frozen samples were sent to several laboratories in a later distribution. Five of the six sets of frozen samples distributed in 1966 were not satisfactory (Table 7); the fat would not redisperse in the samples after they were thawed.

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